ARMY RESEARCH LABORATORY

Variation in Strength of an Unconventional CH···O Hydrogen Bond in an Engineered Protein Cavity

by Rabi A. Musah, Gerard M. Jensen, Robin J. Rosenfeld,
Duncan E. McRee, David B. Goodin, and Steven W. Bunte

ARL-TR-1826 October 1998

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

Citation of manufacturer's or trade names does not constitute an official endorsement or approval of the use thereof.

Destroy this report when it is no longer needed. Do not return it to the originator.

Abstract

We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of CH···O interactions between the ligand and protein. Our study shows that the strength of CH···O interactions can be modulated by over 1 kcal/mol by changes in the C–H band polarity. Consequently, several such interactions may play a significant role in the stability of macromolecular structures.

Acknowledgments

The authors acknowledge C. L. Brooks III, C. D. Stout, M. R. Ghadiri, J. Rebek, Jr., D. A. Case, Y. Cao, S. K. Wilcox, P. Beroza, and B. N. Dominy for their helpful discussions and critical reading of this report. Support for this work was provided by grants GM-41049 (to D. B. Goodin), GM-1744 (to R. A. Musah), and GM-48495 (to D. E. McRee) from the National Institutes of Health (NIH). S. W. Bunte acknowledges financial support from the U.S. Army Research Laboratory (ARL) Director Research Initiative Program. Support was provided by a grant from the Department of Defense (DOD) High-Performance Computing (HPC) Center, ARL, for time on the Silicon Graphics Inc. (SGI) Power Challenge Array (PCA).

INTENTIONALLY LEFT BLANK.

Table of Contents

INTENTIONALLY LEFT BLANK.

List of Figures

List of Tables

INTENTIONALLY LEFT BLANK.

1. Introduction

While hydrogen bonds of the type XH···Y (X, Y = N and/or O) are essentially found in all macromolecular structures, the role of CH···O hydrogen bonds in such systems is uncertain. Recent reports [1–5] have suggested that a significant number of the CH···O contacts observed in proteins, RNA, and carbohydrates represent cohesive interactions, which may, in sufficient numbers, be significant to structure, stability, and function. The existence of short intermolecular CH···O interactions is well-established in many small-molecule crystals [6–9]. In cases where the C–H–O angle is approximately linear and the C···O bond distance is less than the combined van der Waals radii (3.3 Å), these interactions have been labeled as hydrogen bonding. Theoretical calculations have estimated the CH···O bonding interactions to be worth 1–2 kcal/mol [2, 10, 11]. However, many computational approaches utilizing force-field parameterizations do not explicitly consider such interactions as attractive, and CH···O hydrogen bonds are not generally considered in the analysis of structures. The strength of such interactions should vary considerably depending on the C–H bond polarity and may thus be different for the $C_\alpha H$···O interactions observed in some protein β-sheets than those for others involving the more acidic $C_\epsilon H$ protons of histidine [1]. While there is a correlation of the C···O distance with the C–H bond acidity for a number of organic compounds [6, 12–14], nothing is known experimentally about the strength of these interactions and how this strength varies with the polarity of the proton donor.

2. Background

We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of CH···O interactions between ligand and protein. The cavity created by the W191G mutation of cytochrome *c* peroxidase (CCP) has been shown to bind a number of cationic heterocyclic compounds [15–17]. Two such compounds, 2,3,4-trimethylthiazole (234TMT) and 3,4,5-trimethylthiazole (345TMT) bind to the

W191G cavity in essentially isosteric conformations (Figure 1).* Crystal structures of the protein soaked in solutions containing these compounds show clear evidence for binding in the omit electron density maps. No significant differences were observed in the structure of the protein between the ligand-free and either of the ligand-bound states. Placement of a ligand model within the omit density implies a close contact between the C5 ring proton of 234TMT and one carboxylate oxygen of Asp-235. An analogous interaction involving the C2 ring proton is observed for 345TMT. Asp-235 is observed to hydrogen-bond to other cavity-binding ligands and helps determine the cation bonding specificity of this cavity [15, 17, 18]. Thus, the absence of standard hydrogen-bond donors in 234TMT and 345TMT evidently results in the substitution of weaker alternative interactions that fulfill a similar role.

The geometries of the interactions implied by the crystal structures are consistent with a CH···O hydrogen bond [8]. The C···O distances between the ligands and the Asp-235 carboxylate oxygen were 2.95 and 2.85 Å for 234TMT and 345TMT, respectively, shorter than the combined van der Waals radii of C and O (3.3 Å). The C–H–O angles estimated from placing the geometrically optimized ligands† into the electron density were approximately 150° and 140° for 234TMT and 345TMT, respectively (Figure 2). The deviations of these angles from an ideal linear CH···O hydrogen bond are not outside the range (130–170°) of those observed in organic crystals [8]. Although the two compounds differ structurally only by the interchange of the N3 and C4 atoms in the thiazole ring, this difference serves to increase the acidity of the C2 proton in 345TMT

---

* Crystal structures of W191G soaked in 50-mM 234TMT or 345TMT were determined as previously described [17]. For each ligand, the orientation was unambiguous due to density observed for the methyl substituents and for the sulfur atom at the higher contour level. The maps were constructed by merging F_{obsd} for the soaked crystal with F_{calcd} from models of the W191G empty cavity. The structures of 234TMT and 345TMT bound to W191G have been submitted to the Brookhaven database (entries 1ac4 and 1ac8, respectively). Diffraction statistics for 234TMT and 345TMT, respectively, included unit cell parameters (103.76, 73.31, 44.64 Å and 106.03, 75.89, 51.13 Å), resolution (2.0 and 2.1 Å), $I/\sigma_{I(av)}$ (18.2 and 12.4), $I/\sigma_{I(last\ shell)}$ (2.00 and 1.76), number of reflections (18,219 and 19,056), percent completeness (80 and 87%), and R_{sym} (0.048 and 0.062).

† Ligand partial charges were calculated as Kollman electrostatic potential (ESP) charges from geometry-optimized structures using density functional theory. The calculations were performed *in vacuo* as previously described [19] using the program Gaussian 94, Becke3LYP functional, and 6-31G* basis set. ESP partial charges were used to generate an ESP grid with the program Delphi (MSI) using a uniform dielectric constant of 1, ionic strength of 0.0, and full Coulombic boundary conditions. This potential grid was mapped onto the solvent-accessible surface of the molecule calculated with a 1.4-Å probe radius as a color spectrum from +135 kT/e (red) to +200 kT/e (blue).

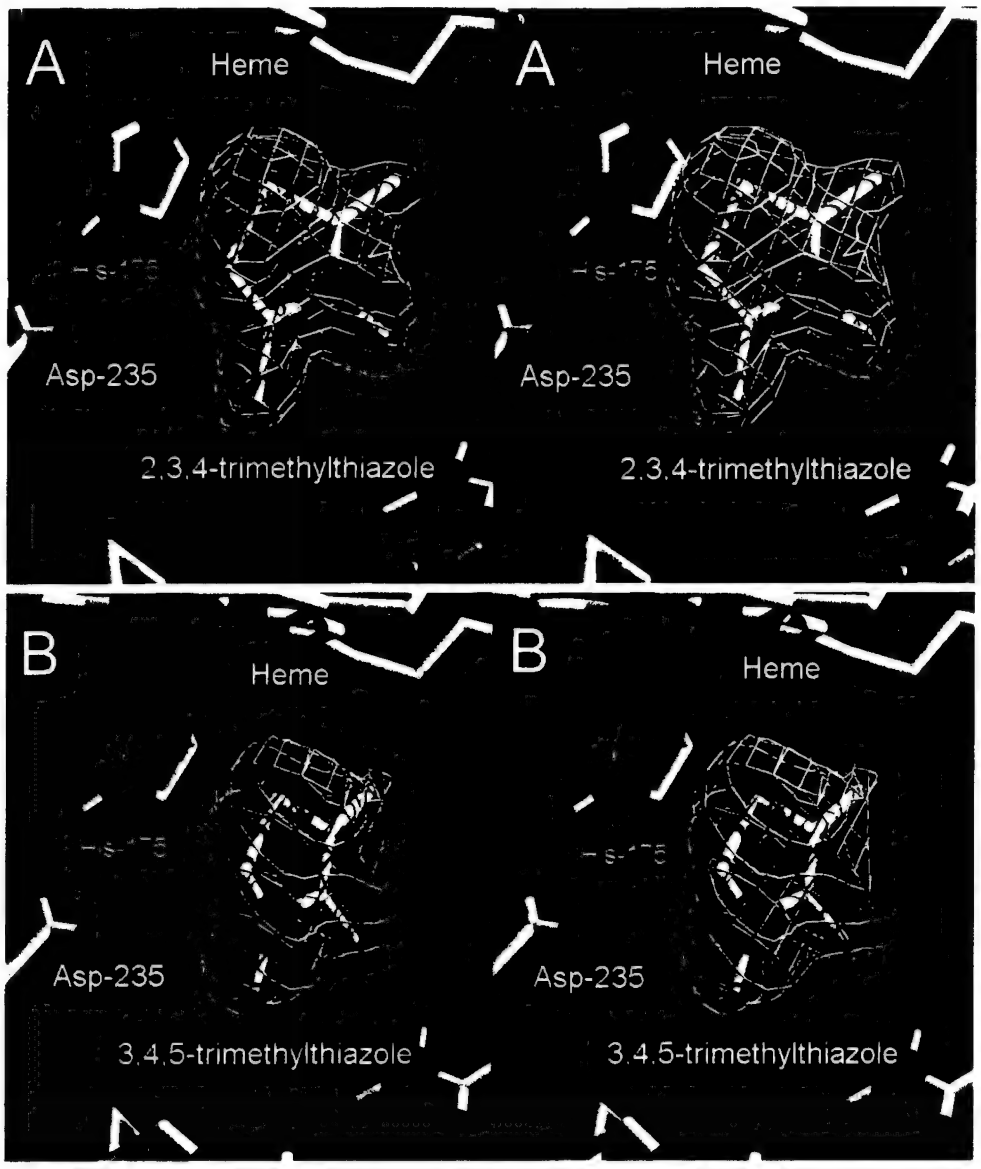
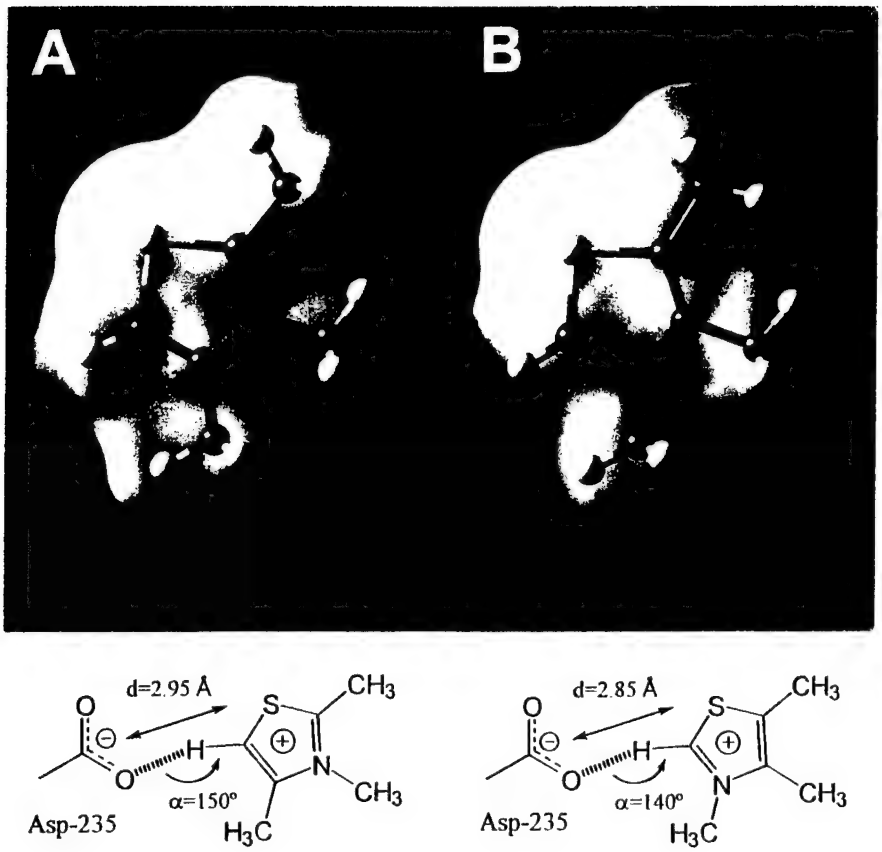


Figure 1. Electron Density for (A) 234TMT and (B) 345TMT Binding to the W191G Cavity. Shown Are Stereoviews of F_o–F_c Fourier Omit Maps Contoured at +4 σ (White) and +9 σ (Red) Superimposed on a Model of the Ligand That Was Placed Into the Omit Density. (See Footnote [*] on p. 2.)

($pK_a \approx 17$–19) [20, 21] relative to the C5 proton in 234TMT ($pK_a \approx 25$–30). This difference in acidities is manifested in the electrostatic potential calculated for the two compounds and mapped onto the solvent-accessible surface (Figure 2). Since the CH···O hydrogen bond is primarily



2,3,4-trimethylthiazole

3,4,5-trimethylthiazole

Figure 2. Electrostatic Potential Surfaces for (A) 234TMT and (B) 345TMT. The Color Map Illustrates the Localized Differences Resulting From the Increased Polarity of the C2–H Proton for 345TMT Relative to the C5–H Bond of 234TMT. (See Footnote [†] on p. 2.) At the Bottom Are the Geometries of the Interactions With Asp-235 That Are Inferred From the Placement of the Geometry-Optimized Ligand Into the Omit Electron Density.

electrostatic in nature, the calculated potentials indicate that 345TMT should form a significantly stronger interaction with Asp-235.

Thermodynamic parameters of 234TMT and 345TMT binding to W191G were determined by isothermal titration calorimetry (Table 1). The results show a five-fold decrease in K_d for 345TMT relative to 234TMT, corresponding to 1.2 kcal/mol of additional binding energy for 345TMT. Due

Table 1. Thermodynamic Parameters for Trimethylthiazole Binding to the Buried Cavity of W191G*

	K_d (mM)	ΔH (kcal mol^{-1})	ΔS (cal mol^{-1} K^{-1})	ΔG (kcal mol^{-1})
234TMT	1.50 (0.16)	−14 (0.6)	−33 (2)	−3.9 (0.1)
345TMT	0.20 (0.03)	−15 (0.7)	−34 (3)	−5.1 (0.2)

to the absence of structural changes in the protein, the similar contacts made with the protein, and the fact that both compounds desolvate the cavity to the same degree, the observed difference in the free energy of binding for the two compounds should result from differences in the electrostatic interactions between ligand and protein and from differences in their desolvation energies. However, estimates of ligand desolvation energies were very similar (Table 2), indicating that the observed difference in binding free energy for the two compounds can be attributed to protein-ligand interactions.

Table 2. Calculated Electrostatic Contribution to the Relative Binding Energy of 234TMT and 345TMT to the W191G Cavity*

	$-\Delta V_{Q\mu}$	$-\Delta V_{Q\alpha}$	$-\Delta V_{QL}$	$-\Delta V_B$	$-\Delta V_{tot}$	$-\Delta G_{solv(water)}$
234TMT	−56.88	3.82	−6.44	−7.26	−66.76	−26.112
345TMT	−57.83	2.98	−5.26	−7.13	−67.24	−26.096
$\Delta\Delta G_{345TMT-234TMT}$	−0.95	−0.84	1.18	0.13	−0.48	−0.016

NOTE: Values are given in kcal/mol.

The electrostatic contribution to the ligand-protein interaction was estimated for the two ligands bound to the protein cavity. Partial charges were calculated for each ligand (Figure 2) to account for

* Dissociation constants and enthalpies were measured at 25° C in 100 mM Bis-Tris propane pH 4.5 by isothermal titration calorimetry (Microcal MC2 ITC calorimeter). W191G (0.2–0.4 mM) was titrated with 5-μL injections of ligand (2–8 mM) equilibrated in the same buffer. Error estimates for K_d and ΔH are given as the standard deviation of multiple determinations, and those for ΔS and ΔG were obtained by propagation.

the different C–H bond polarities, and the protein dipoles/Langevin dipoles (PDLD) method [19, 22]* was used to account for effects of microscopic atomic polarizabilities of atoms surrounding the ligands. The more favorable electrostatic interaction of 345TMT with the protein relative to that of 234TMT (Table 2) arises from both dipole ($\Delta V_{Q\mu}$) and induced dipole ($\Delta V_{Q\alpha}$) terms. These interactions are partially canceled by the solvent screening effects represented in the Langevin grid (ΔV_{QL}) and Born (ΔV_B) terms. The net electrostatic contribution to the binding free energy (ΔV_{tot}) is approximately 0.5 kcal/mol of additional stabilization of 345TMT relative to 234TMT. This value is in good agreement with recent *ab initio* quantum-mechanical analysis of CH···O bond energies [2]. Thus, electrostatics alone form a significant contribution to the observed difference in the binding free energy of 1.2 kcal/mol.

3. Conclusion

In conclusion, the observation of untethered ligand binding to this engineered cavity shows that the CH···O interaction is made by choice and is thus a stabilizing interaction. While it is weak, summation of several such interactions may play a significant role in the stability of macromolecular structures. The strength of this interaction can be modulated by over 1 kcal/mol by changes in the C–H bond polarity. Thus, selective inclusion of polar CH···O interactions in refinement algorithms for nuclear magnetic resonance (NMR) and crystal structures may be justified.

---

* Electrostatic calculations were performed using the PDLD method with the program POLARIS [22]. ΔV_{tot} is the sum of four terms, $\Delta V_{Q\mu}$, $\Delta V_{Q\alpha}$, ΔV_{QL}, and ΔV_B. $\Delta V_{Q\mu}$ is the classical electrostatic interaction of the ligand charges with all of the charges on all protein atoms. $\Delta V_{Q\alpha}$ is the interaction energy from the dipole moments induced by the electric field as a result of atomic polarizabilities. ΔV_{QL} is the energy of interaction of the ligand with the field defined by the solvent dipoles. ΔV_B is the Born energy of interaction with bulk solvent beyond the radius used to define the Langevin dipole grid. Values of ΔV have the sign of electrostatic potential, and, thus, a more positive value for ΔV corresponds to a more negative ΔG. POLARIS calculations were performed with Asp-235 and Arg-48 charged, and all other amino acid residues netural. An estimate of the electrostatic interaction of the ligand charges with the solvent reaction field ($\Delta G_{solv(water)}$ for the two compounds was obtained with a continuum dielectric model (Delphi, MSI) using $\epsilon_{water} = 80$ and $\epsilon_{internal} = 2$, with a grid spacing of 0.25 Å.

4. References

1. Wahl, M. C., and M. Sundaralingam. *Trends Biochemical Science.* Vol. 22, pp. 97–102, 1997.

2. Ornstein, R. L., and Y.-J. Zheng. *Journal of Biomolecular Structure Dynamics.* Vol. 14, 1997.

3. Bella, J., and H. M. Berman. *Journal of Molecular Biology.* Vol. 264, pp. 734–742, 1996.

4. Derewenda, Z. S., U. Derewenda, and P. M. Kobos. *Journal of Molecular Biology.* Vol. 241, pp. 83–93, 1994.

5. Derewenda, Z. S., L. Lee, and U. Derewenda. *Journal of Molecular Biology.* Vol. 252, pp. 248–262, 1995.

6. Sutor, D. J. *Nature.* Vol. 193, pp. 68–69, 1962.

7. Hamilton, W. C., and J. A. Ibers. *Hydrogen Bonding in Solids.* New York: Benjamin, 1968.

8. Taylor, R., and O. Kennard. *Journal of American Chemical Society.* Vol. 104, pp. 5063–5070, 1982.

9. Steiner, T., and W. Saenger. *Journal of American Chemical Society.* Vol. 114, pp. 10146–10154, 1992.

10. Kollman, P., J. McKelvey, A. Johansson, and S. Rothenberg. *Journal of American Chemical Society.* Vol. 97, pp. 955–965, 1975.

11. Seiler, P., G. R. Weisman, E. D. Glendening, T. Weinhold, V. B. Johnson, and J. D. Dunitz. *Angewandte Chemie, International Edition, English.* Vol. 26, pp. 1175–1177, 1987.

12. Desiraju, G. R. *Journal of Chemical Society, Chemical Communications.* Vol. 3, pp. 179–180, 1989.

13. Desiraju, G. R. *Accounts of Chemical Research.* Vol. 29, pp. 441–449, 1996.

14. Desiraju, G. R. *Journal of Chemical Society, Chemical Communications.* Vol. 6, p. 454, 1990.

15. Fitzgerald, M. M., M. L. Trester, G. M. Jensen, D. E. McRee, and D. B. Goodin. *Protein Science.* Vol. 4, pp. 1844–1850, 1995.

16. Fitzgerald, M. M., R. A. Musah, D. E. McRee, and D. B. Goodin. *Nature and Structural Biology.* Vol. 3, pp. 626–631, 1996.

17. Fitzgerald, M. M., M. J. Churchill, D. E. McRee, and D. B. Goodin. *Biochemistry.* Vol. 33, pp. 3807–3818, 1994.

18. Miller, M. A., G. W. Han, and J. Kraut. *Proceedings of the National Academy of Science, U.S.A.* Vol. 91, pp. 11118–111122, 1994.

19. Jensen, G. M, D. B. Goodin, and S. W. Bunte. *Journal of Physical Chemistry.* Vol. 100, pp. 954–959, 1996.

20. Washabaugh, M. W., and W. P. Jencks. *Biochemistry.* Vol. 27, pp. 5044–5053, 1988.

21. Washabaugh, M. W., and W. P. Jencks. *Journal of American Chemical Society.* Vol. 111, pp. 674–683, 1989.

22. Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions.* New York: Wiley-Interscience, 1991.

NO. OF COPIES ORGANIZATION

2 DEFENSE TECHNICAL INFORMATION CENTER
DTIC DDA
8725 JOHN J KINGMAN RD
STE 0944
FT BELVOIR VA 22060-6218

1 HQDA
DAMO FDQ
DENNIS SCHMIDT
400 ARMY PENTAGON
WASHINGTON DC 20310-0460

1 OSD
OUSD(A&T)/ODDDR&E(R)
R J TREW
THE PENTAGON
WASHINGTON DC 20301-7100

1 DPTY CG FOR RDE HQ
US ARMY MATCOM
AMCRD
MG BEAUCHAMP
5001 EISENHOWER AVE
ALEXANDRIA VA 22333-0001

1 INST FOR ADVNCD TCHNLGY
THE UNIV OF TEXAS AT AUSTIN
PO BOX 202797
AUSTIN TX 78720-2797

1 DARPA
B KASPAR
3701 N FAIRFAX DR
ARLINGTON VA 22203-1714

1 NAVAL SURFACE WARFARE CTR
CODE B07 J PENNELLA
17320 DAHLGREN RD
BLDG 1470 RM 1101
DAHLGREN VA 22448-5100

1 US MILITARY ACADEMY
MATH SCI CTR OF EXCELLENCE
DEPT OF MATHEMATICAL SCI
MAJ M D PHILLIPS
THAYER HALL
WEST POINT NY 10996-1786

NO. OF COPIES ORGANIZATION

1 DIRECTOR
US ARMY RESEARCH LAB
AMSRL D
J W LYONS
2800 POWDER MILL RD
ADELPHI MD 20783-1145

1 DIRECTOR
US ARMY RESEARCH LAB
AMSRL DD
J J ROCCHIO
2800 POWDER MILL RD
ADELPHI MD 20783-1145

1 DIRECTOR
US ARMY RESEARCH LAB
AMSRL CS AL TA
2800 POWDER MILL RD
ADELPHI MD 20783-1145

3 DIRECTOR
US ARMY RESEARCH LAB
AMSRL CI LL
2800 POWDER MILL RD
ADELPHI MD 20783-1145

ABERDEEN PROVING GROUND

4 DIR USARL
AMSRL CI LP (305)

INTENTIONALLY LEFT BLANK.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1998	Final, Jan 96 - Jan 97

4. TITLE AND SUBTITLE

Variation in Strength of an Unconventional CH···O Hydrogen Bond in an Engineered Protein Cavity

5. FUNDING NUMBERS

1L161102AH43

6. AUTHOR(S)

Rabi A. Musah,* Gerard M. Jensen,* Robin J. Rosenfeld,* Duncan E. McRee,* David B. Goodin,* and Steven W. Bunte

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

U.S. Army Research Laboratory
ATTN: AMSRL-WM-BD
Aberdeen Proving Ground, MD 21005-5066

8. PERFORMING ORGANIZATION REPORT NUMBER

ARL-TR-1826

9. SPONSORING/MONITORING AGENCY NAMES(S) AND ADDRESS(ES)

10.SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

*Department of Molecular Biology, MB8, The Scripps Reseach Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We have utilized the ligand binding properties of a buried cavity created in the interior of a protein to obtain direct information about the variation in the strength of CH···O interactions between the ligand and protein. Our study shows that the strength of CH···O interactions can be modulated by over 1 kcal/mol by changes in the C–H band polarity. Consequently, several such interactions may play a significant role in the stability of macromolecular structures.

14. SUBJECT TERMS

hydrogen bond, ab initio, calculations, electrostatic modeling

15. NUMBER OF PAGES

15

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

INTENTIONALLY LEFT BLANK.

USER EVALUATION SHEET/CHANGE OF ADDRESS

This Laboratory undertakes a continuing effort to improve the quality of the reports it publishes. Your comments/answers to the items/questions below will aid us in our efforts.

1. ARL Report Number/Author ARL-TR-1826 (Musah) Date of Report October 1998

2. Date Report Received _____

3. Does this report satisfy a need? (Comment on purpose, related project, or other area of interest for which the report will be used.) _____

4. Specifically, how is the report being used? (Information source, design data, procedure, source of ideas, etc.) _____

5. Has the information in this report led to any quantitative savings as far as man-hours or dollars saved, operating costs avoided, or efficiencies achieved, etc? If so, please elaborate. _____

6. General Comments. What do you think should be changed to improve future reports? (Indicate changes to organization, technical content, format, etc.) _____

CURRENT ADDRESS

_____
Organization

_____
Name E-mail Name

_____
Street or P.O. Box No.

_____
City, State, Zip Code

7. If indicating a Change of Address or Address Correction, please provide the Current or Correct address above and the Old or Incorrect address below.

OLD ADDRESS

_____
Organization

_____
Name

_____
Street or P.O. Box No.

_____
City, State, Zip Code

(Remove this sheet, fold as indicated, tape closed, and mail.)
(DO NOT STAPLE)

DEPARTMENT OF THE ARMY

OFFICIAL BUSINESS

NO POSTAGE
NECESSARY
IF MAILED
IN THE
UNITED STATES

BUSINESS REPLY MAIL
FIRST CLASS PERMIT NO 0001,APG,MD

POSTAGE WILL BE PAID BY ADDRESSEE

DIRECTOR
US ARMY RESEARCH LABORATORY
ATTN AMSRL WM BD
ABERDEEN PROVING GROUND MD 21005-5066